JOURNAL OF BACTERIOLOGY, Feb. 1973, p. 711-717

Vol. 113, No. 2
Printed in U.S.A.

Protein Synthesis and Deoxyribonucleic Acid-Membrane Attachment During Thymineless Death in *Escherichia coli*

FRANCES DANKBERG AND DONALD J. CUMMINGS
University of Colorado Medical Center, Denver, Colorado 80220

Received for publication 4 November 1972

The proteins synthesized during thymineless death in *Escherichia coli* B and B/r were analyzed by polyacrylamide gel elctrophoresis. It was found that the amount of a protein of molecular weight 80,000 to 88,000 is greatly increased during thymineless death compared to the amounts of other cell proteins. A technique for the isolation of cell membrane-deoxyribonucleic acid (DNA)-nascent ribonucleic acid (RNA) complex on detergent crystals was used to determine whether DNA might be detached from the cell membrane as a result of thymineless death. It was found that under no conditions of thymineless death or immunity to thymineless death was there any change in the attachment of DNA or pulse-labeled RNA to cell membrane.

When thymine-requiring strains of *Escherichia coli* are deprived of thymine, there is a permanent loss of colony-forming ability. This phenomenon, termed thymineless death (TLD), was first discovered by Barner and Cohen in 1953 (1). Concomitant with cell death, ribonucleic acid (RNA) and protein synthesis continued, and it was possible to induce the synthesis of some enzymes with or without thymine present (4). It was found by Maaløe and Hanawalt (14) that inhibition of protein and RNA synthesis at the time of thymine starvation produced a small but persistently surviving fraction of cells that were termed "immune" to TLD. The size of the immune fraction could be increased by inhibition of protein and RNA synthesis for various periods of time before as well as during thymine deprivation. It was therefore suggested that immunity to TLD might represent cells at a certain stage of deoxyribonucleic acid (DNA) replication, specifically, between replication cycles. However, Cummings and Kusy (6) found, using synchronously growing cultures of *E. coli* B and B/r, that there was no position in the cell cycle at which the bacterial population was more or less immune to TLD. Others have found that immunity to TLD may be detected in the presence of RNA and protein synthesis by varying the growth media and the thymine concentration during growth (9).

Various other factors have been suggested as the killing event in TLD. Thymine deprivation has been shown to cause premature initiation of DNA chains (17), and replication returned to normal after one doubling time. Some groups (18, 19, 24) have reported finding single-strand breaks and other damage in DNA after thymine starvation, but Freifelder and Maaløe (10) found no apparent physical damage to DNA associated with thymine deprivation. Recently, Hanawalt et al. (11) have found one or two single-strand breaks present in bacterial DNA in the absence of thymine but not when chloramphenicol was present. Many of the effects of thymine starvation parallel those of ultraviolet (UV) irradiation and nalidixic acid treatment. Because of the similarity between TLD and UV-induced death, the DNA repair mechanism has been implicated in TLD. The lethal event may be associated with the repair of single-strand nicks in DNA. However, the sensitivities of various mutants to TLD, UV irradiation, and nalidixic acid treatment form no consistent pattern which can be explained by a single lesion in the DNA repair mechanism (7, 8). The importance of continuing protein and RNA synthesis in TLD was investigated further by Cummings and his co-workers (5, 6) using *E. coli* B. Whereas most *E. coli* strains (e.g. B/r, 15, K-12) had a 50-min lag period after thymine deprivation before death was evident, *E. coli* B began losing colony-forming ability immediately and was therefore

termed "sensitive" to TLD (7). It was found that inhibition of protein synthesis in this strain led to inhibition of TLD, immunity to TLD, and recovery following TLD (5, 6).

The possible involvement of the DNA replication cycle has focused attention on the synthesis of specific proteins, particularly membrane proteins, since DNA is believed to be attached to *E. coli* cell membrane. Masker and Eberle (16) have reported that some proteins bind preferentially to DNA and that in thymine-starved cells some of these proteins were found in reduced amounts. Shapiro et al. (20) have found a temperature-sensitive mutant of *E. coli* that is unable to initiate DNA synthesis at the restrictive temperature. Acrylamide gel electrophoresis of membrane proteins showed that one protein (molecular weight = 60,000) in particular was decreased at the restrictive temperature. These findings, however, do not explain the effect of inhibition of protein synthesis on TLD; the synthesis of one or more proteins appears to be necessary for cell death. A recent report by Taketo and Kuno (22) showed that a peculiar protein accumulated in *E. coli* during thymine starvation and nalidixic acid treatment which had the capacity to mask single-stranded phage DNA with respect to its infectivity and endonuclease sensitivity. Inouye and Pardee (12) have reported an increased amount of membrane protein when DNA synthesis was blocked either by thymine starvation or by nalidixic acid treatment.

The synthesis of specific proteins under conditions of thymine starvation, UV irradiation, and nalidixic acid treatment has been investigated in our laboratory by using the technique of acrylamide gel electrophoresis. The possible involvement of the attachment of DNA to the cell membrane during TLD was also investigated.

MATERIALS AND METHODS

Bacterial strains. Thymine-requiring mutants of *E. coli* B and B/r, and a mutant of *E. coli* B requiring arginine, uracil, and histidine as well as thymine (strain DC 103) were used throughout; growth and filtration of these bacteria have been described previously (5, 6).

Labeling E. coli proteins. Total cell proteins in *E. coli* were labeled in some experiments by incubation for 40 to 60 min with ^{14}C-leucine (1 μCi/ml) (New England Nuclear) in the presence of 15 μg of cold leucine/ml. Incorporation was linear for 1 hr under these conditions. In some cases 30 μg of nalidixic acid (Mann) per ml in 0.01 N NaOH was added to the growth media (5, 6). Proteins were also pulse-labeled at various times during growth or TLD by the addition of 1 μCi of ^{14}C-reconstituted protein hydrolysate (Schwarz/Mann) per ml for 5 min. At the end of the labeling period, the cells were killed with NaCN, washed, and suspended in 0.5 ml of 0.03 M tris(hydroxymethyl)aminomethane hydrochloride (Tris) buffer, *p*H 7.5. They were made fragile by the addition of lysozyme, and the *p*H was adjusted to 6.8 by the addition of 50 μliters of 1.25 M Tris, *p*H 6.8. In later experiments the buffer system was changed to 0.0625 M Tris, *p*H 6.8, throughout the procedure. Samples were lysed by the addition of sodium dodecyl sulfate (SDS) to a final concentration of 1% and were stored at −20 C.

UV irradiation of E. coli B. Cells were grown and then transferred into minimal salt medium lacking both glucose and thymine. They were irradiated as described (8) for 2.5 min, after which the viable cell count was less than 1% of original. Glucose and thymine were then added back to the culture, and ^{14}C-amino acid mixture (1 μCi/ml) was added immediately or after 10 min of incubation at 37 C for a 5-min pulse.

SDS-acrylamide gels of labeled proteins. The procedure for SDS slab gels was essentially that of Laemmli (13) and Studier (21). Samples of labeled *E. coli* cell lysates were suspended in a final concentration of 1% SDS, 0.0625 M Tris (*p*H 6.8), 5% mercaptoethanol, 10% glycerol, and 0.001% bromophenol blue. They were then heated at 95 C for 90 sec and applied to 10% or 15% acrylamide gels, with a Tris-glycine buffer system, and subjected to electrophoresis at 26 to 28 v for 18 hr. Gels were stained in 0.125% Coomassie brilliant blue in acetic acid-water-methanol (1:5:5) for 2 hr and destained for at least 24 hr in 5% methanol, 7.5% acetic acid.

Gels were dried according to the procedure of Maizel (15) and placed on Kodak Blue Brand X-ray film for 2 to 14 days.

To determine the relation between mobility and molecular weight, the following protein standards were run with every gel: bovine serum albumin, molecular weight 68,000; trypsin, molecular weight 23,500; and ovalbumin, molecular weight 43,000. A standard curve was obtained by plotting molecular weight versus relative mobility R_F on semilogarithmic paper, and the approximate molecular weight of *E. coli* proteins was determined from this curve. Autoradiogram protein density was estimated by using a Beckman Analytrol microdensitometer. Relative densities of the various protein bands were estimated by cutting out the areas within the peaks from the densitometer tracings and weighing on a Mettler balance.

M-banding procedure. At the end of the appropriate period of TLD, duplicate 15-ml samples of cells were killed by the addition of 0.5 ml of 0.2 M NaCN at 0 C. The cells were centrifuged and washed once with 0.03 M Tris, *p*H 7.5. The pellets were resuspended in 1 ml of 20% sucrose in 0.03 M Tris (*p*H 7.5) and incubated at room temperature for about 5 min. The cells were lysed by treatment with egg white lysozyme (final concentration 1 mg/ml) and ethylenediaminetetraacetic acid (EDTA) (final concentration 0.6 mM), followed by precipitation on

Mg^{2+}-Sarkosyl crystals (23), in which case lysis has been shown to be greater than 99%. Occasionally, the lysozyme treatment was omitted and the cells treated with the detergent without Mg^{2+} for several minutes, followed by addition of Mg^{2+} and formation of crystals. In this case lysis was greater than 97%. The lysozyme-treated cells were incubated at room temperature for 20 to 30 min, followed by the addition of 1.0 ml of 5% sucrose in 0.02 M $MgCl_2$. The cells were layered over a sucrose step gradient consisting of 12 ml of 15% sucrose layered over 10 ml of 45% sucrose, in 0.01 M Tris (*p*H 7), 0.001 M $MgAc_2$, 0.1 M KCl. Sarkosyl NL-30 (30% solution, CIBA-Geigy) was added to the cell suspension to a final concentration of 0.5 to 3% and gently mixed with the cells on top of the gradient. Mg^{2+}-Sarkosyl crystals began to form at 0 C. The gradient was centrifuged at 20,000 rev/min in a Spinco SW25 rotor for 20 min. Detergent crystals banded at the 45% sucrose interface. Gradient fractions were collected and precipitated overnight with cold 10% trichloroacetic acid. Fractions were collected and washed on Whatman glass fiber papers GF/A and dried at 37 C, and 0.5 ml of NCS (Amersham/Searle) was added and allowed to stand several hours at room temperature. Samples were counted in 10 ml of toluene phosphor (2,5-diphenyloxazole, 4 g; and 1,4-bis [2-(4-methyl-5-phenyloxazolyl)] benzene, 50 mg; per liter toluene [Amersham/Searle]). DNA was labeled with either ^{3}H-thymine or ^{3}H-thymidine (New England Nuclear), 0.1 to 0.3 μCi/ml for 45 min. Membrane was labeled with ^{14}C-oleic acid (ICN) in 0.25% "Tween," 0.008 to 0.017 μCi/ml for 45 min. For RNA labeling experiments, cells were incubated with ^{14}C-uracil (New England Nuclear) for 3 min and chased for the indicated times with 30 μg of cold uracil/ml.

Isolation of ^{3}H-labeled high-molecular-weight DNA. *E. coli* B cells were labeled for 45 min during log-phase growth with ^{3}H-thymidine, centrifuged, and washed once with Tris as in the M-band procedure. The cells were resuspended in 0.01 M Tris (*p*H 7.5), 1.2 M NaCl, 0.01 M EDTA and were lysozyme-treated as previously described. Sarkosyl was added to a final concentration of 0.3% to lyse the cells. The lysed cell suspension was extracted three times with two volumes of chloroform-isoamyl alcohol (10:1) to remove protein and was dialyzed overnight against 0.01 M Tris (*p*H 7), 0.01 M EDTA at 4 C. The dialyzed sample was treated with deoxyribonuclease-free ribonuclease (Sigma), 10 μg/ml, at room temperature for 30 min, extracted once more, and dialyzed again. The preparation was still quite viscous at the end of the second dialysis, indicating high-molecular-weight DNA. It was stored in Tris-EDTA, but before use was dialyzed against Tris buffer to remove the EDTA. The final preparation contained about 1% of the total cell protein, 3% total cell RNA, and 60% of the DNA in the crude lysate.

RESULTS

Proteins synthesized during TLD. *E. coli* B was labeled with ^{14}C-amino acids under various conditions of inhibition of DNA synthesis. The acrylamide gel patterns (Fig. 1) of

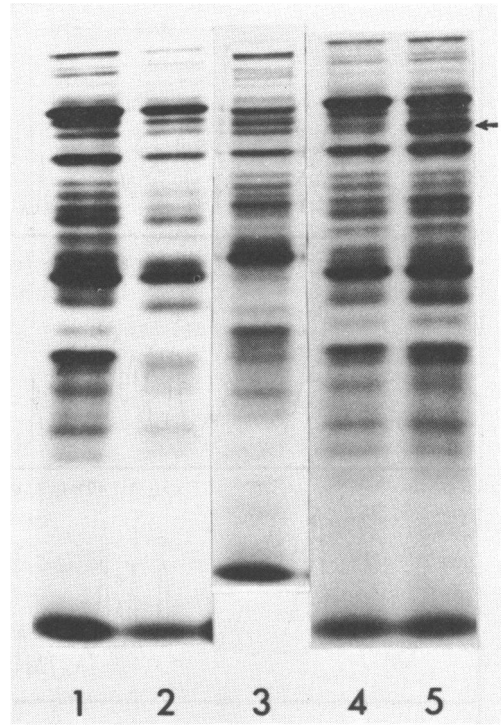

FIG. 1. *Acrylamide gels of E. coli B cell proteins: 1 = 60-min label, log-phase growth; 2 = 60-min label, nalidixic acid treatment; 3 = 5-min pulse label 10 min after UV irradiation; 4 = TLD, 0- to 40-min label; 5 = TLD, 20- to 80-min label. (Arrow designates the protein that accumulated during TLD.)*

proteins labeled during log-phase growth, in the presence of nalidixic acid, or 10 min after UV irradiation appears essentially the same. The pattern of proteins synthesized immediately after UV irradiation is not shown but was the same as the pattern 10 min after irradiation. The pattern of proteins synthesized during the first 40 min of TLD also resembles these. However, later in TLD (20–80 min) it appears as if one band in particular is greatly increased compared to the others. This band appears to be a doublet and corresponds to a molecular weight of 80,000 to 88,000. It should be pointed out that the viable cell count was 0.4% by 40 min of TLD in this experiment, before any substantial buildup of this protein had occurred.

Cultures of *E. coli* B and B/r were pulse-labeled at various times during growth and TLD to determine precisely when this protein was synthesized. Figure 2 shows densitometer tracings of acrylamide gel patterns of proteins from these cultures. It can be seen for both *E. coli* B and B/r that a particular band "C" is increased compared to other proteins synthe-

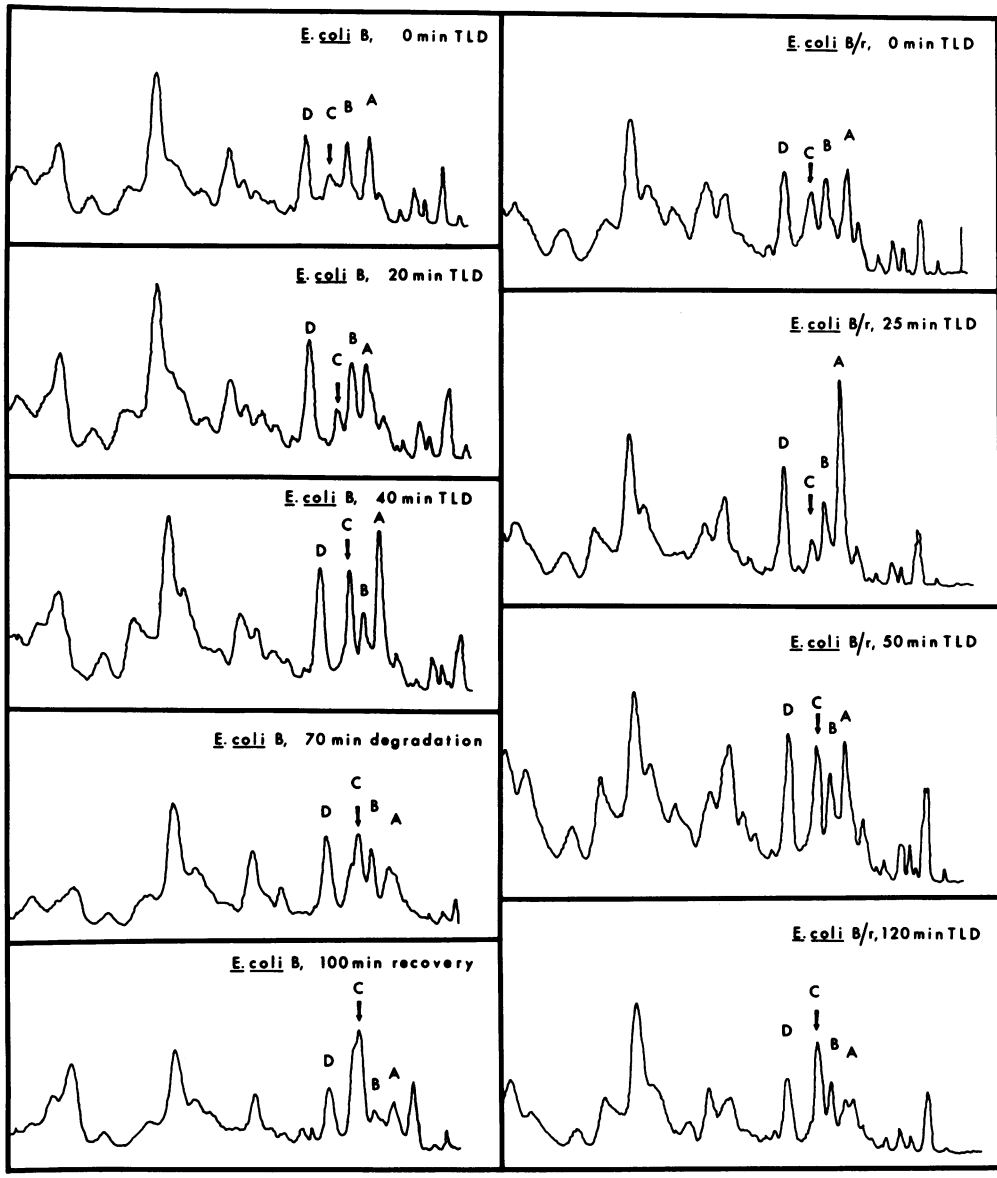

FIG. 2. *Acrylamide gels of E. coli B and B/r cell proteins pulse-labeled at various times during TLD, recovery from TLD, and degradation after TLD.*

sized at 40 to 50 min after TLD. This band corresponds in position to the band visibly increased in the long-term labeling experiments (Fig. 1). It must be remembered that *E. coli* B loses viability immediately, but strain B/r does so after a 50-min lag period.

To quantitate these results, we have arbitrarily chosen to compare the amount of protein in band C with the three other major bands near it, namely A, B, and D. This comparison can only be approximate due to such factors as the arbitrary choice of a base line for excising the areas, the limits of saturation of the X-ray film, and the sensitivity of the densitometer. The most striking changes occur in the relative intensities of bands B and C; the pattern is similar, however, if C is compared with A or D. For *E. coli* B the band ratio C/B changes during 0, 20, and 40 min of TLD from 0.8 to 0.4 and then up to 1.4. For *E. coli* B/r this ratio becomes at 0, 25, 50, and 120 min of TLD, respectively, 0.9, 0.4, 1.1, and 1.9. In other

experiments the ratio was as high as 3 or 4 after 50 to 55 min of TLD. It can be seen from Fig. 2 that the amount of protein C appears first to decrease early in TLD, and then increases out of proportion to the amounts of other proteins later in TLD.

The effect of the readdition of thymine on the synthesis of protein C was investigated. Since protein synthesis could not be inhibited for these experiments (5), there was no increase in viable cell count during the first 100 min after the addition of thymine. Cultures previously allowed to suffer 50 to 55 min of TLD were pulse-labeled at various times after thymine was restored. It can be seen from Fig. 2 that the addition of thymine did not reduce the relative amount of protein C synthesized. The band ratio C/B, which was 3.2 after 50 min of TLD in this experiment, remained 3.2 at 100 min after the readdition of thymine.

To see if the degradation of protein C was affected by the addition of thymine, *E. coli* B was pulse-labeled after 55 min of TLD and then transferred into fresh, unlabeled medium containing thymine for 70 min. The C/B ratio, which was 1.6 after 55 min of TLD, was unchanged. Figure 2 shows that this protein was still a major cell component after 70 min in unlabeled thymine medium.

No other consistent changes in 10% acrylamide gel patterns were observed during TLD. To examine the low-molecular-weight proteins, the samples were examined on 15% acrylamide gels, and no further changes were observed.

Attachment of DNA to cell membrane. When cells were labeled with ^{3}H-thymine and ^{14}C-oleic acid during log-phase growth, it was found that 80 to 95% of the ^{3}H label and 80 to 90% of the ^{14}C label appeared in the M-band (detergent) fraction of the gradient. Occasionally a precipitate was found at the bottom of the 45% sucrose layer which was similar in appearance to the detergent crystals and contained both ^{3}H and ^{14}C label. It was believed to be of essentially the same composition as the M-band, and the radioactivity in this precipitate was included in the M-band fraction for all calculations. It may have been a result of overloaded gradients, and the qualitative results were the same with or without this fraction. The remaining 10 to 15% of the radioactivity was at the top of the gradient.

Cells labeled with ^{3}H-thymine and ^{14}C-oleic acid were extracted three times with butanol, and the aqueous and organic phases were sampled and counted in toluene phosphor. It was found that 71% of the ^{3}H appeared in the aqueous phase, and 87% of the ^{14}C in the organic phase, indicating that the ^{3}H-thymidine was incorporated into a water-soluble cell component (DNA) and the ^{14}C-oleic acid into a lipid-soluble (membrane) component. High-molecular-weight DNA, labeled with ^{3}H-thymidine and isolated as described in Material and Methods and mixed with ^{14}C-oleic acid-labeled cells before being applied to the gradient, did not adventitiously bind to the membrane-detergent complex more than about 36%, whereas about 82% of the ^{14}C-membrane was attached to the complex. When DNA was sheared by drawing up the lysed cells 10 to 15 times in a 25-gauge needle before application to the sucrose gradient, the amount of DNA attached to the M-band was reduced from 71 to 40%, and the ^{14}C-membrane component was reduced from 77 to 51%.

The effect of TLD on DNA-membrane attachment is summarized in Table 1. Cells labeled with ^{3}H-thymine and ^{14}C-oleic acid during log-phase growth were transferred into thymineless medium and sampled at 0, 30, and 60 min of TLD for *E. coli* B and at 0, 120, and 150 min for strain B/r. During this time viable cell count was reduced to 1.6 to 3% of original for B, and to 29 to 58% of original colonies for B/r. In no case was there any change in the percentage of labeled membrane or attached DNA which appeared bound to detergent. Since TLD did not appear to result in release of DNA from cell membrane, the possibility that the immune state is a result of a temporary detachment of DNA-membrane was considered. To investigate this question strain DC 103 was used (5, 6). By incubating these cells in a medium that lacked all requirements, or by preincubation in medium containing thymine but lacking arginine, uracil, and histidine, protein and RNA synthesis could be inhibited, and a population immune to TLD was produced. *E. coli* strain DC 103 was grown and labeled during log-phase growth with ^{3}H-thymine and ^{14}C-oleic acid as usual and transferred into thymineless medium either with or without arginine, uracil, and histidine or transferred into medium containing thymine but without arginine, uracil, and histidine for 100 min of preincubation followed by transfer into thymineless medium as indicated in Table 1. Samples were taken at various times after preincubation and after filtration into TLD medium, and M-bands were isolated. The viability of the cultures under the different regimes varied from 0.6% of the 0-min colonies to 53%, but in no case was there any change in the percent of ^{3}H-DNA which remained attached to membrane-detergent complex.

TABLE 1. *Effect of TLD on DNA-membrane attachment*

Bacterial strain	Treatment	Per cent viable cell count	Per cent counts/min in M-band[a]	
			^{3}H-DNA	^{14}C-membrane
E. coli B	0 min TLD	100	86	79
	30 min TLD	50	90	81
	60 min TLD	2	90	85
	DNA sheared		40	51
	Extraneous DNA added		36	82
Strain DC 103	70 min (−Thy, Arg, Ura, His)	30	87	87
	70 min (−Thy, +Arg, Ura, His)	1	83	81
	Preincubation with Thy for 100 min then 0 min TLD	100	91	85
	100 min TLD	53	89	83
E. coli B/r	0 min TLD	100	88	77
	120 min TLD	58	95	88
	150 min TLD	29	82	75

[a] Most values represent the mean of two experiments.

Attachment of RNA to DNA-membrane; effect of TLD and the immune state. By virtue of its attachment to DNA template, newly synthesized RNA should be found associated with the M-band DNA-membrane complex (23). It was found in our experiments that log-phase *E. coli* B cells, pulse-labeled with ^{14}C-uracil for 3 min and chased with unlabeled uracil, repeatedly gave M-band fractions containing about 42% of newly synthesized RNA. The remaining radioactivity was spread throughout the gradients. Cells pulse-labeled during log-phase growth and then transferred into thymineless medium for up to 60 min of TLD, or pulse-labeled after 60 min of TLD, showed no significant changes in the percentage of RNA attached to DNA. Pulse-chase experiments, in which cells were labeled while in thymineless medium at 0 min of TLD and after 30 min of TLD and then chased with cold uracil for 0 min or 30 min after pulse-labeling, also showed no significant changes in RNA-DNA-membrane attachment (32 to 46% of radioactivity was in the M-band fraction).

Similar experiments in which chloramphenicol (20 μg/ml) was used to inhibit protein synthesis during TLD and produce an immune state merely increased the percentage of ^{14}C-RNA found in all M-band fractions; cells pulsed during log-phase growth, at 0 min of TLD or at 30 min TLD with a 0-min chase or 30-min chase with cold uracil, all had between 69 and 85% of the radioactivity in the membrane-detergent complex. There was no significant effect of TLD or of immunity to TLD on RNA-DNA attachment.

DISCUSSION

Our results show that after thymine starvation a protein of 80,000 to 88,000 daltons was increased with respect to the synthesis of other cell proteins. The pulse-labeling experiments, however, indicated that the accumulation of this protein is not evident until late in TLD, and well after substantial cell death has occurred. The accumulation did not appear to be related to the strain-dependent characteristics of TLD such as the lag period exhibited by *E. coli* B/r and not by *E. coli* B. It is possible, therefore, that this protein is not the killing agent, but accumulates as a result of thymine deprivation and cell death. Perhaps thymine is required as a cofactor to convert this protein to another one which is necessary for cell viability, or perhaps in more than the normal amounts this protein can mask DNA. Another possibility, suggested by Inouye and Pardee (12), is that without thymine the intracellular location of this protein is changed. For example, if it is normally a membrane component but is found in the cytoplasm when thymine is lacking, its effect may be to mask DNA and cause cell death. When thymine was deprived in the culture, the amount of synthesis of this protein was first decreased and then increased. This may represent an attempt by the cell to delay the accumulation of this protein by temporarily shutting off its synthesis. It could also be that the effect of thymine deprivation is not on masking DNA, but on cell division rather than DNA replication. For example, formation of septum or other parts of the

apparatus necessary for equal division of the cell membrane into daughter cells may be inhibited by this protein. Our results are different from those of Inouye and Pardee (12) in that we found that only TLD and not nalidixic acid or UV irradiation produced the increased synthesis of a protein. They also differ in that their protein "X" responded rapidly to inhibition of cell division, whereas the change we found occurred late in TLD. It is possible that our techniques cannot detect the small changes in the concentration (or location) of this protein which may cause cell death; however, from our data the accumulation of this protein may be a correlate rather than a cause of TLD. The recent findings of Hanawalt et al. (11) suggest that endonuclease activity is enhanced during TLD, and it may be that the protein C described here plays such a role. Alternatively, this protein may have ribonucleoside diphosphate reductase activity; this enzyme has been shown to be derepressed during thymine deprivation (2) and consists of two subunits of 180,000 and 78,000 molecular weight (3). Finally, it is not clear whether protein C is a breakdown product of another cellular protein; the similarity of the results obtained by long-term labeling and pulse-labeling experiments make this unlikely but do not exclude it. Further work is required to clarify these possibilities.

The effect of TLD on the attachment of DNA to cell membrane was also investigated and under no conditions of TLD, with or without protein synthesis, and with or without an immune fraction, were there any changes in the apparent attachment of DNA or RNA to membrane.

ACKNOWLEDGMENT

The work represented here was supported by Public Health Service grant no. AI 06472 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. Barner, H. D., and S. S. Cohen. 1954. Induction of thymine synthesis by T2 infection of a thymine requiring mutant of *Escherichia coli*. J. Bacteriol. **68**:80–88.
2. Biswas, C., J. Hardy, and W. S. Beck. 1965. Release of repressor control of ribonucleotide reductase by thymine starvation. J. Biol. Chem. **240**:3631–3640.
3. Brown, N. C., Z. N. Canellakis, B. Lundin, P. Reichard, and L. Thelander. 1969. Ribonucleoside diphosphate reductase. Purification of the two subunits, proteins B_1 and B_2. Eur. J. Biochem. **9**:561–573.
4. Cohen, S. S., and H. D. Barner. 1954. Studies on unbalanced growth in *Escherichia coli*. Biochemistry **40**:885–893.
5. Cummings, D. J., and A. R. Kusy. 1969. Thymineless death in *Escherichia coli:* inactivation and recovery. J. Bacteriol. **99**:558–566.
6. Cummings, D. J., and A. R. Kusy. 1970. Thymineless death in *Escherichia coli:* strain specificity. J. Bacteriol. **93**:1917–1924.
7. Cummings, D. J., and L. Mondale. 1967. Thymineless death in *Escherichia coli*: deoxyribonucleic acid replication and the immune state. J. Bacteriol. **102**:106–117.
8. Cummings, D. J., and A. L. Taylor. 1966. Thymineless death and its relation to UV sensitivity in *Escherichia coli*. Proc. Nat. Acad. Sci. U.S.A. **56**:171–176.
9. Deutch, C. E., and C. Pauling. 1971. Survival and macromolecular synthesis during incubation of *Escherichia coli* in limiting thymine. J. Bacteriol. **106**:197–203.
10. Freifelder, D., and O. Maaløe. 1964. Energy requirement for thymineless death in cells of *Escherichia coli*. J. Bacteriol. **88**:987–990.
11. Hanawalt, P. C., A. R. Grivell, and H. Nakayama. 1972. Correlation of DNA strand breaks and their repair with transcription in *Escherichia coli*. IV. International Congress on Biophysics, Moscow. E III c 1/3. p. 208.
12. Inouye, M., and A. B. Pardee. 1970. Changes of membrane proteins and their relation to deoxyribonucleic acid synthesis and cell division of *Escherichia coli*. J. Biol. Chem. **245**:5813–5819.
13. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
14. Maaløe, O., and P. C. Hanawalt. 1961. Thymine deficiency and the normal DNA replication cycle. I. J. Mol. Biol. **3**:144–155.
15. Maizel, J. V., Jr. 1971. Polyacrylamide gel electrophoresis of viral proteins. Methods in Virology **5**:179–246.
16. Masker, W. E., and H. Eberle. 1971. DNA replication and proteins that bind to DNA. Proc. Nat. Acad. Sci. U.S.A. **68**:2549–2553.
17. Pritchard, R. H., and K. G. Lark. 1964. Induction of replication by thymine starvation at the chromosome origin in *Escherichia coli*. J. Mol. Biol. **9**:288–307.
18. Reichenbach, D. L., G. E. Schaiberger, and B. Sallman. 1971. Effect of thymine starvation on chromosomal structure of *Escherichia coli* JG-151. Biochem. Biophys. Res. Commun. **42**:23–29.
19. Reiter, H., and G. Ramareddy. 1970. Loss of DNA behind the growing point of thymine-starved *Bacillus subtilis* 168. J. Mol. Biol. **50**:533–548.
20. Shapiro, B. M., A. G. Siccardi, Y. Hirota, and F. Jacob. 1970. On the process of cellular division in *Escherichia coli*. II. Membrane protein alterations associated with mutations affecting the initiation of DNA synthesis. J. Mol. Biol. **52**:75–89.
21. Studier, F. W. 1972. Bacteriophage T7. Science **176**:367–376.
22. Taketo, A., and S. Kuno. 1972. Accumulation of a "DNA-masking protein" in *E. coli* under inhibitory conditions of DNA synthesis. J. Biochem. **71**:497–505.
23. Tremblay, G. Y., M. J. Daniels, and M. Schaechter. 1969. Isolation of a cell membrane-DNA-nascent RNA complex from bacteria. J. Mol. Biol. **40**:65–76.
24. Walker, J. R. 1970. Thymine starvation and single-strand breaks in chromosomal deoxyribonucleic acid of *Escherichia coli*. J. Bacteriol. **104**:1391–1392.